

(This Y64 stock is substantially L)

$$\begin{array}{r} 874 \\ \times 377 \\ \hline 1251 \end{array}$$

$\approx B_1 / -B_1$, ca 690:302 = 2.25
Lee, V.

Empire 524
184:75 = 2.42

$T(0)$.

$-R$	$-S$	$+R$	$+S.$
7	8	1	4
5	13	0	7
5	8	1	10
5	13	2	4
<hr/>			
22	42	4	25
			/ 93

$$K_1^2 = < 1.$$

no difference in distributions

$T(B_1)$.

$$\begin{array}{r}
 8 & 6 & 3 & 7 \\
 10 & 12 & 1 & 4 \\
 5 & 17 & 1 & 4 \\
 2 & 3 & 0 & 3 \\
 7 & 13 & 2 & 3 \\
 \hline
 32 & 41 & 7 & 21 & /101.
 \end{array}$$

B_1	ϕ_C	L_{ac}	V
-	+	-	R
+	-	+	S.

54 83 11 46 /194

$$\begin{array}{ccc}
 \uparrow & \uparrow & \nearrow \\
 +s & -s & -R \\
 23 & 43 & 29
 \end{array}$$

in %: 29 43 6 23.

$-S/-R$ should be same as before.

Colony description

53.

May 28, 1947.

Structure various cultures in EMB:

58-161 A24 (16h.)
L+S ca 1:5 → all S.
→ all L.

Y40 (do.) purify to Co^L and Co^S lines.

Y87 all S. ?? → S.

Y53 all L? Pick 1 large → all L? some S?? →

Y10 L+S ca 10:1. Purify. → L OK.
S → somewhat different from L.

Y64 (all?) large. Exc.? L → OK. Pick for stock.

Y46. (all?) large. S? → somewhat smaller than Y64L

Y94. Friedman. L. Some small?

L = large "rough"

S = small, "smooth?"

Y40 S from B4 synths. all S.

in EMB, Lact + S do not show a granular
lact + L do., particularly in (Y10).

Take L colonies for new stocks. Label as Co^L and Co^S respectively.

Y40/6. all small.

Y87/6. large (somewhat mucoid) and small. →

(and Y87L). As compare Y87S × Y10L with Y40S × Y94L
58-161S × Y64L.
Some produce less sediment, more pellicle
in broth

Effect of colony dimorphism on segregations

536.

May 29, 1947.

A) Y40- $C_0^S \times Y53. [C_0^L]$

B) Y40- $C_0^L \times Y53. [C_0^S]$.

all T₁^S

This argues for an error in the setting-up of the experiment! Test Y40 suspensions which were kept!

A. Large colony selection:

Lac-	Lac+
17	6
20	4
9	2
20	5
17	5
11	6
<hr/>	
84	28
<hr/>	

Lac-	Lac+
7	5
14	8
17	5
<hr/>	

41	38	15	18	156.
81	84	31	28	112
122	46	168		
<hr/>				

together: 122 : 46 / 168

$$\chi^2 = 9 \left(\frac{1}{38} + \frac{1}{15} + \frac{1}{31} + \frac{1}{81} \right)$$

= .026

.067

.032

.012

$9 \times .137$

= 1.2

$P =$

b). ~~8/19~~

9	8
17	6
13	8
14	8
12	11
16	6
19	2
<hr/>	

16	3
14	7
<hr/>	
30	10

b) 104 100 49⁴⁵ / 149. $\chi^2 = < 1.$

A) 118 122 46⁵⁰ / 168 $P = \dots$

222 / 95 317

May 3, 1947

A. 58-161L x Y64L.

B. 58-161S x Y64L

C. 58-161S x Y64S.

D. [58-161L x Y64S].

Stock test parents:

58-161 S } indistinguishable!
58-161 L }

Y64S } occasional L.
Y64L. } ca 1:1 S:L.

Test parents as T1. [also "Y40" from exp. 536.

a:a' ca 1:10 in frequency.
(T(0):T(B₁)).

B:B' do lower frequency of protogahs. May due to suspensoris.

C very few recombinants. (SxS)

D. same. [Y64S n.g. for recombinants ???].

large gathering of the

55-

June 3, 1841.

$$Y_{100} \times 58-161. [Y_{53-V_{LT}^R} \times 58-161 V_{LT}^S] .$$

plates of V-test too dry for good test.

4.9.

$T(0)$.	$-R$	$-S$	$-R$	-1
	$-R$	$+R$	$+R$	$+1$
	$-R$	$-R$	$-R$	-1
	$+R$	$+S$	$-R$	-1
	$+R$	$-S$	$-R$	-1
	$-R$	$-S$	$-R$	-1
	$+R$	$+S$	$-R$	-1
	$-R$	$-R$	$-R$	-1
	$+R$	$-R$	$-R$	-1
	$+S$	$-R$	$-R$	-1
	$+R$	$-R$	$-R$	-1
	$-S$	$-R$	$-R$	-1
	$+S$	$-R$	$-R$	-1
	$-S$	$-R$	$-R$	-1
	$+R$	$-R$	$-R$	-1
	$-R$	$-R$	$-R$	-1
	$-R$	$-R$	$-R$	-1
		<i>But off.</i>		
		\checkmark		
			$GS:3R.$	

V_{17} is therefore either 30_{rel.} units to the left of TL, just right of V_{15} , or to the right of TL. This could be settled by studying interaction i -loc. This favors the V_{15} -TL position. Or, in the cross $BP \times Y100$, a B loc P change in V_1 signatures would indicate an intercalary location.

T(B₁). ~~~~~

$$T(0) = -R - S + R + S$$

$$\frac{9}{2} \quad \frac{7}{3} \quad \frac{1}{2} \quad \frac{4}{1}$$

<u>Total:</u>	- R	- S	+ R	+ S
	14	22	3	12

$$S = 36/51 = 70\%$$

$$R = \frac{V_{IT} R_s}{T + \frac{V_{IT}}{V_{TL}}} \quad \text{or} \quad b \quad s$$

$$T(B_1) = \frac{1}{2} \quad \frac{3}{9} \quad 0 \quad \frac{2}{5}$$

June 3, 1947.

Reversion of B11. Tryptophane requirement.

Plate ca 10^9 cells/plate of B11 into $\text{F}^{(0)}$

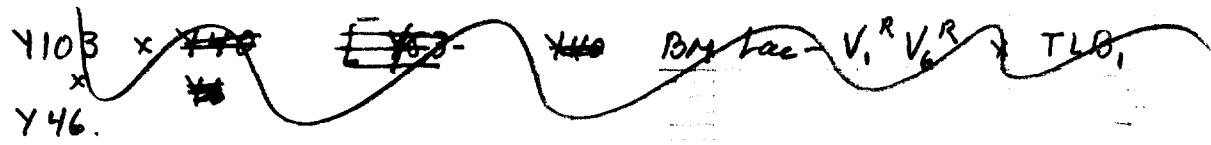
on one plate, ca. a dozen colonies on surface. (~~possibly~~ contamination).

Recom. test with phage

∴ no colonies

Segregation of V_6 , V_1

539



V103 x V10

no T_6 sensitivities seen; phage probably n.g. (see tests)
for T_1 and Lac:

$T(0)$	-R	-S	+R	+S.
2	0	4	6	
2	0	2	7	
2	0	4	4	

6 0 10 18

$T(0,1)$	0	3	8	
2	1	8	3	
2	0	4	6	
0	0	0	4	
9	0	3	7	
2	0	5	2	
2	0	3	2	

13 1 26 32

OK.

19 1 36 49.

Sex activity of sonates.

540.

Prepare Y40, Y53 suspensions as usual in ~~BB~~ saline. Treat in same apparatus for 1 hour. X=Hg; C=control. Add $\frac{1}{2}$ + $\frac{1}{2}$ + plate $\frac{1}{2}$ ml.

a) Y40X in N.A.

do. 1:100 $\rightarrow 10^4$

b). Y53X in N.A.
do 1:100 $\rightarrow 10^{+4}$

c). Y40X \times Y53C ++

d). Y40C \times Y53X ++

e) Y40X + Y53X ++

f.) Y40C \times Y53C. ++

g.) (Y40C \times Y53C) + (Y53X \times Y40X) tabed ++

544

in the middle $\frac{V_2}{2}$.

June 4, 1947.

T2R - slide received from Lucia. Size = 10⁹ when made.

plate covered & colonies

Cla - ficate

542

Plate 58-161 on NA + Cla:

12 h.

- | | |
|-------------|---|
| 1. 0 | |
| 2. 200 r/ml | no inhibition |
| 3 400 | colonies somewhat smaller,
no papillation |
| → 4 600 | marked inhibition; no papillae
colonies pinpoint |
| 5 800. | very marked inhibition. colonies minute |

24 h.

v. sl. inhibition:
large and small
colonies.
all single colonies are
small; papillae in
streaks.

36 h.

400

- 600 colony dimorphism  visible papillation
800. colonies very large or very small.  distinct papillation.

Range between 5 - 700 r/ml probably optimal. (See mutagens?)

June 6, 1941

Rec'd from A. Boivin, histiomes C₁, S and C₂, recordates Y105, Y106 respectively.

- a) Test on Cl₄-agar: papillae found; streaked to purify and test for aerogenes.
- b) Test on T(0) - grows well both on liquid + solid T(0).
- c) Fer - both strongly lact
- d) Phage reactions:

	T1	T2	T3	T4	T5	T6	"T7"	
Y105	R	R	R	R	R	R	R	
Y106	R	R	R	R	R	R	R	
K-12	S	S	S	S	S	R	R	! cf. Huehning!

∴ available phages are n.g. find new ones?

Test Cl₄^R types on glucose fermentation tubes. (piles of colonies, plate streaked = colloidal

105 (C ₁)	A	+	+	+	+	+		
	B	-	-	-	-	-	Y108 = C ₁ Cl ₄ ^R . S	

106 (C ₂)	A	-	-	-	-	-	Y108 = C ₂ Cl ₄ ^R . S.	
	B	-	-	-	-	-	Y109 = C ₂ Cl ₄ ^R . S.	

+) Sucrose: no definite fermentation by either. Both show slight papillae in regions of Keool streaks. When these are streaked,

June 10, 1947.

- a) Prepare extract + stink filtrate of a 24 hour culture of Y105 in YB.
 Filtrate: sediment cells in centrifuge; stink - filter.

Extract: suspend cells = ca 200 ml in 10 ml H₂O. Treat sonically for 2 hours at 0°C. Sediment debris + emulsify supernatant with benzene overnight. Remove sediment + excess benzene; remove benzene in vacuo. Should leave a stink preparation. —

Add 1:10 to YB tubes for assay.

b). Inoc Y107, 108, 109 into glucose-gas tubes + 1:1 filtrate.

• 24 h.	107	—
	108	—
	109	—
	<u>—</u>	no acid

c) Inoc 107, 108, 109 into YB + filtrate. grow 24 h. Use this to inoculate mainly gas tubes: 6 h.

(Transformation: !)	107	—
	108	—
	109	±

d. Streak out 109; 109/Fb; 109/Fc; 109/Fc/test on Cl₄ plates to detect sensitive colonies.

(a 20% serial dilution series was used! sensitive is noted!)

e. Dextrose - all dextrose - on second transfer on EMB plates.

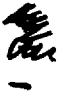
Transformation

545

June 13, 1947.

Y109. in glucose broth  gas
 ||
 ||
 ||

Y109 in YB.
then glucose -
 -

Y109 in YB + 1:1 filtrate of
Y105  suspicious test for T.

Y109 in YB + 1:10 extract
of Y105 +
 +
 ±
1:10 extract Y105 - no acid.

∴ It appears very likely that a transformation has
been effected from Ac^- to Ac^+ .

June 11, 1947.

Inoculate a watered suspension of Y106 in dilution 1:1000.
Inoculate into YB; incubate 24 hours. Plate onto EMM lactose.
ca $500 \times 58 =$ ca 30,000 colonies examined.

No clear-cut, smooth, Lac- seen. (possibly due to transformation-reversion.)

About 8 possible, small-colony Lac-? were made for test.

Also pick a number of small colony types in hopes of finding an R₂ transformable to S₁.

Pick 40 overall 10 large colonies to small tubes of YB, and sort on basis of "autoagglutination".

a) Large colo: all disperse 10/10.

b) small colo. 23 clumped 17 disperse. Discard disperse
and moi. clumped types into large YB tubes for further tests.
In general, growth of these types is poorer.

of 23 clumped in 1st test, 15 do not show leucine growth
on second in large tubes.

4 good roughs 546-1, 2, 3, 4

Treat washed suspension of Y106 with 4.5% Na desoxycholate (DX) for 3 hours at room temp. Slight lysis observed. Wash & resuspend into 10 ml YB broth + 1 mg. desoxyribonuclease. Incubate overnight and plate for mutant detection. P21.

A23 - look over for preliminary small colonies. Overlay with:
agar 1.5%, N2 case 2%; Y. Extr. 1% MA23. The colonies of this

O red.
11 picked

form are extremely uniform, and less
than .1% of the original sample are
abnormally small, e.g. / .

now. <.1% are ±

Examine for small colonies.

ca 250 colonies/plate \times 26 = 6,500 tests.

P23. X red 16 picked

A24. 1 red * picked. To small YB.

Grew:

O
X
Δ.

Tests on T/0

spread treated suspension on EMB lactose. 82 \times 10 cols. = 8000
examined

T(0)

Δ	1	-	Y110
	2	-	Y111
	3	-	Y112
	4	-	Y113
	5	-	Y114
	6	-	<u>Y115</u>
	7	++	
	8	-	Y116

others grown on minimal
or second test or
wt E. coli by L.R.

valine; isoleucine

X 11 - Y117 arginine

12 ± Y118 arginine

13 ++ Y119 arginine

14 ++ Y120 valine isoleucine

15 - Y121 cystine

16 - Y122

17 - Y123

18 ++ Y124

O 31 ++

32 - Y106 ++

Y106 ++

∴ at least 18/24 mutants.

$$18/8000 = .5\%$$

Requirements identified by L. Rodriguez
Harris Designes

Later:

133 lysine }
138 leucine } from 118 ∴ arginine -
139 histidine }

141, 142, 143: arginine from 121: cystine.

[June 20]

Chemical test for uronide:

Take 10 ml. suspension of S_1 + S_2 ; sediment and suspend in 60% HCl + 1:10 1% alcoholic neptunocyanol. Boil 1 min. Let cool; add 1 ml ether shake & examine.

C_1 - red color at surface

C_2 - no color.

Both show a green fluorescence in aqueous phase.

Test for Sucrose fermentation

a) on plates (EMB). - C_1 + C_2 both negative

b) in liquid - C_1 , C_2 , Y109 all negative
after several days.

Acetate utilization:

after 4 days - Y109 \equiv
Y106 \pm

DX-alcohol procedure.

June 23, 1947.

26 hour culture of Y105 : 600 ml YB in 1 liter flask.
shake at 30° .

Sediment + suspend in 20 ml 4.5% DX. add benzene and
shake at 25° from 1230P23 to .

P23. Sediment and remove debris + ~~the~~ benzene phase
by filtration.

~~add 10 vols. 100% Alcohol.~~ — Collect sediment in a sterile
tube.

Sedimentation required ca 5 hours, supernatant collected.
due to thick emulsion. Possibly pH too low

Upon addition of alcohol, a thick floccous ppt. formed. Probably consists
largely of desoxycholic. Sediment and resuspend in alcohol to dissolve
desoxycholate. Sediment (easily done in centrifuge). Supernatant
ppts in aqueous 6.8 buffer. probably desoxycholate
^{same DX in solution.}

Try dissolving sediment in H₂O. OK - very viscous solution.

R₂. In second p₂₃ attempt, add a few drops of NaOH to prevent p₂₃ of
NaDX + alcohol.

Test R₁; R₂ on Y109.

negative.

no gas + produced.

sterility - OK.

(Repeat, omitting DX)

June 26, 1947.

Y109 in YB + 545 extract.

tests on glucose tubes.

sterility	-
Y109	-; -
Y109+TP	+ +

transformation OK.

streak out on Cl_a.

Preparation of TP: alcohol procedure.

Autolyse 500 cc² Y105 in 15 ml NaCl 9% + 1 ml benzene at 50°.
Sediment + separate extract.

500-X1 aliquot centrifuging free of cells. Shake with benzene + store overnight at cold room.

500-X2 Add 6 vols. 100% alc. to extract. Ppt ca 5-10 mg of material.
sediment transfer to sterile tube; sediment suspended in 100% alcohol
for 3 hours. Sediment and residue in H₂O. 1 ml = ca. 25 ml culture.
Add 1:10 to YB tubes to test for sterility and activity.
Inoc with Y109. After 16 hours, add 1/2 ml culture to glucose tests.

1. Y109	-	elution moi \bar{z} X _L and 109!
2. Y109+X ₂	+	
3. Y 109+X ₂	++	
4. Y109+X ₂ +1mg DNase	-	
5. Y 109+X ₂ +1mg DNase	+	
6. sterility control. moi. \bar{z} 109 in vac. \pm		

Transformation.

June 27, 1947.

1. Y109
2. Y109 + X₂
3. Y109 + X₂
4. Y109 + X₂ + 1 mg DNase
5. Y109 + X₂ + 1 mg DNase
6. Y109 + X₁
7. Y109 + X₁
8. X₁.
9. X₂.

Preparations from 550.

June 21-28 1947.

Prepare extract from 500ml \circ Y105 by alcohol pot. method,
after toluene autoclaving 3 1/2 hours. Add water to 10 ml YB.

	+ 8 hours test
1. 109	+++
2. 109 + X	+++
3. 109 + X	+++
4. 109 + X + DNase	+++
5. 109 + X + DNase	++
6. X.	not sterile?

- non very slow growth on transfer to glucose test.

P28. Add 10 vols alcohol to remainder of X to sterilize. 1 pt + stored in
in 70% alcohol.

"109" inorulum ?? probably in error. - Leibovitz: